INHIBITION OF RESPIRATION, GERMINATION, AND GROWTH BY SUBSTANCES ARISING DURING THE DECOMPOSITION OF CERTAIN PLANT RESIDUES IN THE SOIL[1]

Z. A. PATRICK AND L. W. KOCH[2]

Abstract

Substances capable of markedly inhibiting the respiration, germination, and growth of tobacco seedlings were obtained after residues from timothy, corn, rye, or tobacco plants had been allowed to decompose under appropriate conditions in the soil. Bio-assay tests, based on manometric methods, made it possible to determine some of the conditions under which the decomposition gave rise to phytotoxic by-products and to obtain rapidly an accurate measure of the degree of their toxicity. Among the factors influencing the production of the toxic products, the species and stage of maturity of plant material added, the water content and pH of the soil, and length of decomposition period seemed most important. Substances capable of reducing the respiration of tobacco seedlings by 50 to 90% were consistently obtained when any of the above-mentioned plant residues had been decomposing for 15 to 25 days under conditions of high soil moisture and when the pH of the soil solution was below 5.5 throughout this period. Timothy gave rise to substances possessing the highest toxic activity, followed by corn, then rye, and finally tobacco. Aqueous extracts of unamended soil or of macerated plant tissues prepared either before decomposition had taken place or when decomposition was inhibited by autoclaving the soil were not toxic.

The toxic substances, obtained by water extraction, exhibited an inhibiting effect on respiration of tobacco seedlings after an exposure of less than one hour and also induced darkening and necrosis of root cells. Some extracts affected the cells of the apical meristem most severely while others affected only the cells of the elongation region.

While the toxic substances have not been identified, they were relatively nonspecific in their action, affecting tobacco, timothy, and barley in approximately the same manner and in many instances possessed also antifungal activity. They were soluble in water, insoluble in petroleum ether, stable in acid, and most active in the pH range 4.5 to 5.8. They were precipitated by alkali and the activity was markedly reduced when readjusted to the acid range. They were also heat stable and did not lose their activity in storage at 1°–3° C. provided they were covered by a layer of toluene.

Because of their rapid production and the marked injurious effects exerted on various plants it is believed that these toxins may perform a significant role in the field as the primary cause of some root rots and in predisposing plants to attack by organisms not normally regarded as pathogenic.

Introduction

The return of plant residues to the soil has long been recognized as being of agronomic importance especially as related to tilth, and maintenance of organic matter. Such practice, however, sometimes results in deleterious effects on succeeding crops. The precise mechanisms of the injurious effects on plants are not clearly understood. Some investigators attribute the harmful effects of crop residues to the depletion of nutrients or immobilization of nitrogen by soil organisms during the decomposition processes (20, 25). Others have suggested that the harmful effects were mainly due to their influence on the microbiological balance with the resulting effect on the survival and virulence of

[1]Manuscript received May 1, 1958.
Contribution No. 1686, from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2]Plant Pathology Section, Science Service Laboratory, Harrow, Ontario.

many soil pathogens (13, 21, 26). These and other points of view have received much attention over the years and yet much of the information on this subject, which is considered to be a phase of the general problem of soil toxicity, is still contradictory. Thus the actual existence and presumed importance of such naturally occurring soil toxins are still matters of controversy and, although many of the earlier investigators were able to isolate and identify substances toxic to plant growth from certain soils (28), most of their findings were of an indirect nature and the origin of these substances was not definitely established. Relevant literature on the general subject of soil toxicity has recently been comprehensively reviewed by Bonner (8) and Audus (2).

More recent evidence has been more conclusive (4, 9, 14, 16) and there appears to be little doubt that substances toxic to living plants can originate from excretions of roots and underground stems of certain living plants (5, 6, 19, 22), or may be liberated from plant residues during their decomposition (3, 4, 11, 23). Frequently it may not be possible to distinguish between these two sources. However, many of the investigators concerned with crop sequence and the so-called soil sickness effects have generally come to believe that the residues of certain plant species are the most important contributors to soils of compounds toxic to plants.

Although many of the facts concerning soil toxicity, its existence, nature, origin, and function, still remain contentious, it is generally agreed that the incorporation and subsequent decomposition of plant residues in the soil can have important implications not only in the field of horticulture but also to plant pathologists. In this respect also there is an increasing volume of evidence to suggest that many soil toxins arising from plant residues may predispose or condition the plant to invasion by many root-rot-producing organisms (10, 24).

In view of the growing recognition that soil toxins may be playing an important role in the etiology of many root rots (10, 13, 17, 24), as well as their probable importance in connection with certain root disorders of tobacco in Ontario which at present cannot be assigned completely to parasitic organisms, this study was undertaken. The main purpose of the work reported in this paper was to determine whether and under what conditions decomposition of the residues of some of the crops grown in this area would result in the production of substances toxic to plants and to determine the various morphological and physiological changes that such substances induce in the plant in the absence of other causal factors which could modify its susceptibility to attack by some of the root-rot-producing organisms.

Materials and Methods

Crop Residues and Soils Used

Materials from timothy, rye, tobacco, and corn plants were used in the decomposition studies. The soil and plant materials for most of the experiments were obtained from the Science Service field plots in which the above-mentioned four crops had been grown for at least 2 consecutive years. In the

controls the soil used was from the same series of plots except that the plots had been fallow and kept free of plant growth. Soils from the Delhi and other tobacco-growing areas were also used in many of the experiments.

The plant materials which were added to the various soils were obtained at three different stages of growth: young, intermediate, and nearly mature. In the young stage, about 5 to 6 weeks after planting, the plants were 12 to 18 in. high and succulent. In the intermediate stage, 6 to 8 weeks after planting, the plants were 20 to 36 in. high and were close to the flowering stage. At near maturity, 10 to 14 weeks after planting, the corn and tobacco plants were still quite green while the rye and timothy were only partially green in color. Entire plants including the roots were harvested at the stages of growth mentioned, cleansed of soil, cut up into pieces approximately 1 in. long, and were either used immediately or air-dried and stored for future use.

The soil was obtained from the same plots which grew each of the above crops. Soil samples were taken to a depth of 8 in., from four to six locations within each plot. The plants and roots in this layer were separated and the soil made into one composite sample for each plot. The soil throughout all the plots appeared to be a uniform Fox sandy loam with a pH range of 6.2 to 6.8. All plots had received identical fertilizer applications.

Decomposition and Extraction Procedures

To 1000-g. quantities of fresh soil were added 250 g. fresh weight, or 60 g. air-dry weight, of the various plant materials including roots, stems, and leaves; the materials were mixed thoroughly and placed in 1-gal. sterile glazed crocks. Each of the plant materials was added to the same soil in which it had been growing and no plant materials were added to the fallow soil. Water was then added to each of the crocks. In some instances enough water was added to saturate the contents while in others the moisture content was adjusted to approximately field capacity. The term field capacity is used throughout these tests to denote that amount of moisture which is retained in the soil after gravitational drainage of excess water has taken place. This condition was attained by adding water to the soil and allowing the excess to drain away. The crocks were covered with aluminum foil to reduce water loss during the decomposition period and were kept at 60° to 70° F. for periods varying from 0 to 30 days. For each of the series enough crocks were prepared to allow extraction from the contents of two crocks after decomposition periods of 0, 5, 10, 15, 20, 25, and 30 days for each of the crops used. Parallel series of crocks were prepared every 30 days and, over a period of 3 years, 18 such decomposition series were carried out. In another series of tests the soil and plant materials were placed in 2000-ml. Erlenmeyer flasks which were plugged with cotton and autoclaved for 40 minutes at 18 lb. pressure before being allowed to incubate for the various periods.

After each incubation period two crocks of each crop were removed and 1000 ml. of water added; the contents were stirred for 15 to 20 minutes and allowed to settle. The liquid was then decanted and its pH determined.

After being centrifuged at approximately 4500 r.p.m. for 30 minutes, the liquid was then filtered first through an 8-lb., and again through a 16-lb., Seitz filter to remove soil particles, microorganisms, and undecomposed plant debris. The precipitate and remainder of the crock contents were discarded. The filtrates were covered with a layer of toluene, placed in cold storage, and kept at 1–3° C. until used. The various filtrates were tested for phytotoxic properties usually within 5 days after preparation.

Tests for Toxicity

A method, similar to that used to demonstrate the toxic nature of breakdown products from peach-root residues (23), was used in this study as the principal assay method to determine whether the above-mentioned filtrates, containing decomposition by-products of the various plant residues, possessed phytotoxic properties and also to evaluate the relative toxicity of such substances. In this method, which is based on manometric techniques, the respiration rates of tobacco seedlings, previously exposed to aqueous extracts containing the decomposition products of the various plant residues, are compared with respiration rates of similar seedlings exposed to aqueous extracts of soils which do not contain such residues or with respiration rates of tobacco seedlings exposed to 0.01 *M* phosphate buffer solution.

The effect of each filtrate on the respiration of tobacco seedlings was tested as follows. Tobacco seed of the variety Harrow Velvet was surface sterilized and placed in Petri dishes containing 1.2% sterile agar. The plates were then placed in an incubator held at 25° C. The seedlings were used 6 days after being sown, at which time they had germinated and grown approximately 6 mm. At this time sterile and apparently uniform tobacco seedlings from at least four Petri dishes were collected and placed in two other dishes containing sterile distilled water. Twenty milliliter portions of each filtrate to be tested (previously adjusted to pH 5.3–5.8 with HCl or NaOH) were placed in sterile Petri dishes, two separate Petri dishes for each filtrate. Into each of the dishes containing the filtrates, 100 of the 6-day-old tobacco seedlings were then placed. The seedlings were then allowed to remain overnight (16 hours) in the various filtrates at room temperature, which was maintained at approximately 20° C. The same number of tobacco seedlings was also placed in dishes containing sterile 0.01 *M* phosphate buffer solution (pH 5.3–5.8) and allowed to remain there for the same period. The next day the tobacco seedlings were removed from each of the filtrates, rinsed in sterile distilled water, and placed in Warburg flasks containing 2.2 ml. of sterile 0.01 *M* phosphate buffer solution. Fifty seedlings were placed in each flask; thus, there were in each instance four flasks with seedlings which had been pre-exposed to the same filtrate. The oxygen uptake of the tobacco seedlings pre-exposed to the various filtrates was then determined in the conventional manner by the direct method of Warburg (30). Filter paper immersed in 0.2 ml. of 20% KOH placed in the center well was used to absorb the CO_2 produced. The oxygen uptake of tobacco seedlings pre-exposed to the phosphate buffer solution was used as the control and given the respiration value of 100%. The

respiration of the tobacco seedlings pre-exposed to the various filtrates was then calculated as percentage of the control. All tests were carried out at 20° C. and readings of the oxygen uptake were taken every hour for 6 hours. Each filtrate was tested on at least three separate occasions. Whenever differences in the O_2 uptake of flasks containing identical filtrates exceeded 15% the tests were repeated.

Additional bio-assay tests were carried out in which the effect of the various filtrates on germination and growth of tobacco, barley, and timothy seed and seedlings was also determined. The test procedure was similar for all three types of seed and only the test with tobacco will be described. Two layers of filter paper were placed in the bottoms of Petri dishes, the walls of which were then lined with absorbent cotton and then these dishes were autoclaved for 15 minutes at 18 lb. pressure. Each of the filtrates to be tested was then apportioned to the dishes in 25 ml. amounts. After the liquid was absorbed by the filter papers and the cotton liner, approximately 100 Harrow Velvet tobacco seeds were sprinkled on the filter paper impregnated with the various filtrates and incubated at room temperature. Results were obtained after 7 days of incubation. In the controls, phosphate buffer solution was added in place of the various filtrates. After the 7-day incubation period 25 seedlings, selected at random, were removed from each dish, placed on glass slides along with a millimeter scale, and photographed with the camera locked in position. After printing, measurements were made of each seedling (see Figs. 11 to 13) and the relative toxicity of the various filtrates was evaluated from this data.

Modifications of the above procedures and additional methods are described below in conjunction with the experiments in question.

Observations and Results

Decomposition of Various Plant Residues in the Soil and the Effect of the Resulting Products on Respiration of Tobacco Seedlings

Effect of Soil Moisture During the Decomposition Period on Relative Toxicity of the Resulting Products

Various investigators have reported that soil toxicity due to organic constituents is mainly associated with soils characterized by poor aeration or excessive moisture conditions (21, 24, 28). Many of the specific organic compounds detrimental to plant growth also have been isolated most frequently from such soils (29). From such results it has been generally concluded that most of the toxic compounds occurring in such soils are formed chiefly during the decomposition of plant residues and other soil organic matter under conditions of high soil moisture. It has not been shown, however, that the substances arising during decomposition of plant residues under conditions of high soil moisture are more toxic to plants than those formed during decomposition under normal moisture conditions or that the concentrations of such substances are different under these conditions. Experiments were therefore carried out

to determine whether the decomposition of similar plant residues, in essentially identical soils but under different moisture conditions, would give rise to substances which would exert different effects on the respiration of tobacco seedlings. In the first series of tests the various plant materials were allowed to decompose in soils held at two moisture levels, saturation and field capacity. For the tests, two series of crocks were prepared simultaneously, soil and plant materials from the same composite samples being used. To one series of crocks sufficient water was added to saturate the contents, while in the other the moisture level was adjusted to field capacity by allowing the excess water to drain away. Both series were then kept at 60°–70° F. for 20 days. At that time water extracts were obtained from each of the crocks and the effect of the substances formed during decomposition under the two conditions on the respiration of tobacco seedlings was determined in the manner described earlier. The results are presented in Table I.

TABLE I

OXYGEN UPTAKE OF TOBACCO SEEDLINGS AS AFFECTED BY THE PRODUCTS OF DECOMPOSITION OF PLANT RESIDUES IN SOILS HELD AT SATURATION AND AT FIELD CAPACITY

Plant material added to soil	After 20-day decomposition period at:			
	Saturation		Field capacity	
	pH range*	O_2 uptake†	pH range	O_2 uptake
Soil only	6.4–6.6	128	6.4–6.6	131
Timothy	4.8–5.2	18	5.9–6.4	90
Rye	5.3–5.8	45	6.0–6.6	104
Corn	4.9–5.4	28	6.0–6.5	99
Tobacco	5.5–5.9	68	6.9–7.5	112
Control 125 ($\pm$8)‡				

*pH range after the 20-day decomposition period; pH of each extract was adjusted to 5.3 prior to testing.

†O_2 uptake, in μl. of O_2 after 6 hours at 20° C., of 50 Harrow Velvet tobacco seedlings. In each instance the tobacco seedlings were exposed for 16 hours to the various extracts then returned to phosphate buffer (pH 5.3) before their O_2 uptake was determined. Each figure is based on the average of four different decomposition series for each of which three determinations of four replicates each were made.

‡O_2 uptake, in μl. of O_2 (after 6 hours at 20° C.), of comparable seedlings pre-exposed (16 hours) to 0.01 *M* phosphate buffer solution; based on the average of 12 determinations.

From the results in Table I it will be noted that, when the two above-mentioned soil moisture levels were maintained during the decomposition processes, substances were formed which induced different effects on the respiration of tobacco seedlings. Whereas the substances formed when decomposition of plant residues took place in soils under saturation conditions strongly inhibited the oxygen uptake of tobacco seedlings, those formed in soils held at the lower moisture levels had relatively mild inhibiting effects on the oxygen uptake of the tobacco seedlings. Such differences, however, could have been partly due to differences in concentration since the toxic products were not totally eliminated even when the decomposition occurred in soils held at the lower moisture levels inasmuch as the products arising from

the decomposition of the plant residues, especially those from timothy or corn, were still quite toxic. Extracts of soils to which no plant residues had been added, maintained at either of these two soil moisture levels, had no inhibiting effects on the respiration of tobacco seedlings and presumably did not contain toxic substances.

Additional experiments were carried out in which the two soil moisture levels mentioned above were varied during the test period. Decomposition was carried out on the one hand for 15 to 20 days in soils held at field capacity followed by saturation and, on the other, for the same period under saturation conditions followed by a dry period. In general, it was noted that, if decomposition was carried out for 15 to 20 days under moisture levels equal to field capacity followed by flooding, the resulting extracts, obtained 3 to 5 days following the flooding, were highly toxic. On the other hand, if the decomposition was carried out under saturation conditions for the same period then adjusted and maintained at field-capacity moisture levels, the toxicity decreased slowly and was reduced by only one half 10 days after the return to the drier soil conditions.

Effect of Plant Residue and Decomposition Period on Relative Toxicity

Experiments were next carried out to determine the relationship between the type of plant material added to the soil, the decomposition period, and the relative toxicity of the resulting products. In these experiments all plant materials used were at the intermediate stage of growth and enough water was added to saturate the contents of the crocks. Procedures with respect to the preparation of plant materials, decomposition, extraction, and assay were similar to those described earlier.

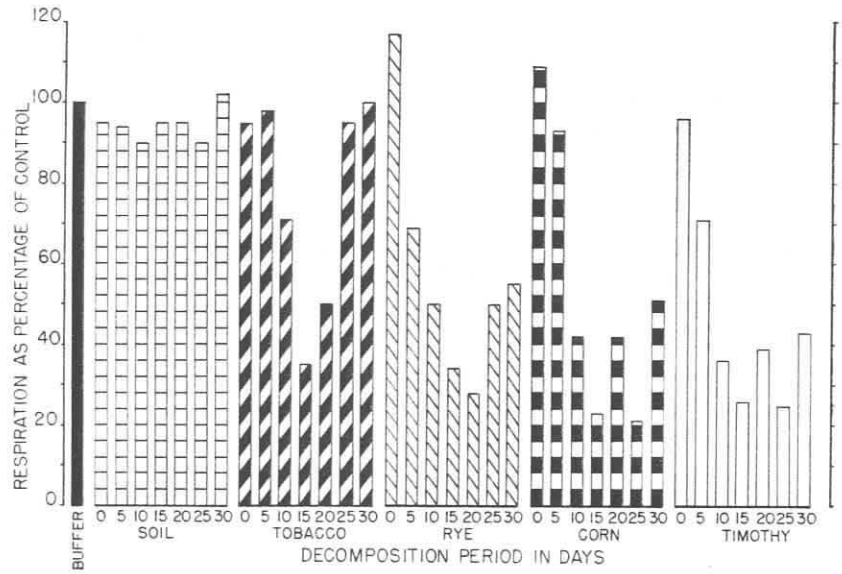

FIG. 1. Relative toxicity, based on percentage inhibition of respiration of tobacco seedlings, as affected by type of plant material added to the soil and period of decomposition.

The results of these experiments are presented in Fig. 1. In these tests the oxygen uptake of tobacco seedlings after they had been exposed for 16 hours to the various extracts was compared with the control, i.e., similar seedlings exposed to 0.01 *M* phosphate buffer solution for the same period of time. The results, calculated as percentages of the control, are based on average results from nine different decomposition series carried out over a 3-year period and involving more than 400 extracts from the various crops.

From the data summarized in Fig. 1 it will be noted that substances which adversely affect the respiration of tobacco seedlings arise during only certain stages of decomposition of the various plant residues. Moreover extracts from fallow soil to which no plant residues had been added had little or no effect on the respiration of tobacco seedlings as was evidenced by the fact that the respiration of seedlings which had been exposed to aqueous extracts from such soils was essentially identical to that of seedlings which had been in the buffer solution for the same period of time. On the other hand, the respiration of seedlings exposed to extracts of soil to which various plant residues had been added varied and depended on the type of residue added to the soil and the length of the decomposition period. When the plant material was added to the soil and extracted within a few hours of preparation, most of the extracts only slightly affected the respiration of tobacco seedlings. Thus, the extracts from tobacco and timothy produced a slight inhibiting effect, while rye and corn produced some stimulation of respiration. After 5 days of decomposition, differences began to appear; extracts containing decomposition products of tobacco or corn still had little effect on respiration whereas extracts of soil in which rye or timothy had been decomposing induced about 30% inhibition of respiration. After the 10-day period, extracts of soil in which timothy, corn, or rye had been decomposing contained substances which had marked inhibiting effects on respiration of tobacco seedlings and extracts of soil in which tobacco had been decomposing also began to exert inhibiting effects at this time. By the time the various plant residues had been decomposing in the soil for 15 days, all extracts, except that of fallow soil, contained substances which were highly toxic and the respiration of tobacco seedlings was markedly reduced when exposed to any of the extracts. After the 20-day decomposition period the toxicity of the extracts still remained high and respiration, in most cases, was inhibited by more than 50%. After the 25-day period, the toxicity of extracts containing decomposition products of tobacco had almost disappeared and the toxicity of rye was somewhat reduced. Extracts of soil in which corn and timothy had been decomposing, however, were still highly toxic. A further reduction in the relative toxicity of most of the extracts took place by the 30th day, although the rate of its reduction was not the same for the various plant materials used. Thus, while extracts of soil in which tobacco had been decomposing for 30 days no longer inhibited the respiration of tobacco seedlings, those containing decomposition products of timothy or corn still inhibited the seedling respiration by about 50%.

Effect of Plant Maturity on Relative Toxicity and Duration of the Toxic Period

In view of the marked changes in the chemical composition of plants during the various stages of growth (15, 20) experiments were next carried out to determine the effect of maturity on degree of toxicity of the decomposition products and relative rate of their production. The various plant materials were added to the soil at three different stages of their development, namely young, intermediate, and mature. Decomposition, as in the previous experiments, was carried out under high soil moisture conditions and the relative toxicity of the resulting products was again based on percentage inhibition of respiration of tobacco seedlings.

The results are summarized in Table II, in which, for ease of comparison, the extracts are divided into four activity categories, nontoxic, mild, intermediate and highly toxic, on the basis of percentage inhibition of respiration they induced. From these results it will be noted that the stage of maturity at which the plant residues were added to the soil was an important factor in determining the time required for toxic substances to be formed, as well as in determining the relative toxicity of these substances. When residues from young plants are added to the soil, substances which inhibit the oxygen uptake of tobacco seedlings are produced during relatively early stages of the decomposition process. Most of these substances exert relatively mild effects on respiration of tobacco seedlings and are inactivated quite rapidly during the subsequent stages of the decomposition. Under laboratory conditions most of the toxic effects disappear by the 25th day of decomposition. When residues

TABLE II

EFFECT OF PLANT MATURITY AND PERIOD OF DECOMPOSITION ON RELATIVE TOXICITY OF THE RESULTING PRODUCTS

Age of plant material added to soil	Type of plant	Decomposition period (days) at 60–70° F.						
		0	5	10	15	20	25	30
Young (5 to 6 weeks after planting)	Soil only	−*	−	−	−	−	−	−
	Timothy	−	+*	++	+++	++	−	−
	Rye	−	−	+	++	+	−	−
	Corn	−	−	+	++	+	−	−
	Tobacco	−	−	+	+	−	−	−
Intermediate (6 to 8 weeks after planting)	Soil only	−	−	−	−	−	−	−
	Timothy	−	+	+++	+++	++	+++	++
	Rye	−	+	++	+++	+++	++	++
	Corn	−	−	++	+++	++	+++	++
	Tobacco	−	−	+	+++	++	−	−
Mature (10 to 14 weeks after planting)	Soil only	−	−	−	−	−	−	−
	Timothy	−	−	−	++	++	+++	+++
	Rye	−	−	−	+	++	+++	++
	Corn	−	−	−	+	++	+++	+++
	Tobacco	−	−	−	+	++	++	−

*Toxicity rating: − =nontoxic: extracts which inhibit respiration of 6-day-old tobacco seedlings by less than 19%; +, ++, +++ =mild, intermediate, and highly toxic, producing inhibition of respiration of 20–40%: 41–60%, and 61–95% respectively.

from plants at the intermediate stage of growth are incorporated into the soil, toxic substances are formed somewhat later in the decomposition cycle but they are more toxic and persist for a longer period of time. When residues from mature plants are added to the soil, toxic products do not arise until during the relatively late stages of decomposition.

Effect of Type of Plant Residue and Decomposition Period on Soil pH and Relative Toxicity of the Resulting Products

Although acidity is known to play an important role in soil toxicity, the relation of acidity to the formation of toxic decomposition products is still not clearly understood. Thus certain plant residues when added to the soil increase soil acidity during their decomposition while others produce very little change (20, 25) and it has been noted that the lowest yield of the succeeding crop was obtained following those plant residues which created the highest acidity. The harmful effects to succeeding plants were considered by some investigators to be due mainly to the unfavorable influence of such plants on the soil reaction (20, 25), while others believed that the harmful effects were mostly due to toxic organic compounds which were produced during the decomposition of plant residues under conditions of high acidity (20, 21). Experiments were therefore undertaken to determine whether some of these questions could be clarified using inhibition of respiration as the index of toxicity. The purpose of the first series of tests was to determine the relationship between the degree of toxicity of the various extracts and their pH. For these tests the soil and plant materials were obtained from field rotation plots mentioned above. The initial pH of the soil ranged between 6.2 and 6.8. At the end of each 5-day period pH of the soil solutions in which the various plant residues had been decomposing was determined. The relative toxicity of the extracts which were obtained at the same time were also determined by means of the tobacco seedling respiration test. The results are summarized in Table III, from which it is evident that, following the addition of the various residues to the soil samples, the pH of the soil or of resulting extracts was not altered appreciably as long as no decomposition had taken place. Likewise the extracts obtained at this zero-day decomposition period exerted no inhibiting effects on the respiration of tobacco seedlings, which in many instances was actually stimulated. As the decomposition period was extended, the pH of the extracts changed considerably, the pH of some extracts having shifted from the 6.2–6.9 range to the 4.8–5.9 range after only a 5-day decomposition period. Corresponding with the change in pH, there was also a change in the relative toxicity of the extracts, inhibition of respiration of tobacco seedlings being obtained only with extracts whose pH was below 6.0 at this time. As the decomposition progressed, more and more extracts were obtained with pH in the 4.8 to 5.9 range. Although decomposition of the same types of plant materials for equal periods of time did not in all instances give rise to substances having the same inhibiting effects on the respiration of tobacco seedlings, there was a high degree of consistency between the relative toxicity of extracts and their pH. Thus, for example, extracts with pH of 4.8 to 5.3 were

TABLE III

EFFECT OF PLANT SPECIES AND DECOMPOSITION PERIOD ON pH AND RELATIVE TOXICITY OF THE RESULTING SOIL EXTRACTS

Decomposition period (days) at 60–70° F.	pH range*	Type of plant material added to the soil									
		Soil only		Timothy		Rye		Corn		Tobacco	
		No. of extracts†	Toxicity rating‡	No. of extracts	Toxicity rating	No. of extracts	Toxicity rating	No. of extracts	Toxicity rating	No. of extracts	Toxicity rating
0	6.2–6.5	20	−	29	−	22	−(s)	24	−(s)	8	−
	6.6–6.9	16	−	7	−	14	−(s)	12	−(s)	16	−
	7.0–7.3	0		0		0		0		12	−(s)
5	4.8–5.3	0		8	++	4	++	2	+	2	+
	5.4–5.9	0		16	+	19	+	6	+	1	+
	6.0–7.0	36	−	12	−	13	−	28	−	33	−
10	4.8–5.3	0		14	++	2	++	10	++	1	++
	5.4–5.9	0		16	++	23	+	17	++	1	+
	6.0–7.0	36	−	6	−	11	−	9	−	34	+
15	4.8–5.3	0		16	+++	10	++	9	+++	6	+++
	5.4–5.9	0		13	++	15	++	21	++	7	++
	5.4–5.9	0		18	++	20	++	20	++	2	++
	6.0–7.5	36	−	11	−(+)(s)	12	−(+)(s)	7	−(+)(s)	34	−(s)
30	4.8–5.3	0		4	+++	2	+++	3	++	0	
	5.4–5.9	0		18	++	16	++	18	++	2	+
	6.0–7.5	36	−	14	−(+)(s)	18	−(+)(s)	15	−(+)(s)	34	−(s)

*pH range of the aqueous extracts obtained after the various plant residues (at the intermediate stages of growth) had been decomposing in the soil for the periods indicated. The pH measurements were made with a Beckman model G pH meter.

†A total of 36 extracts of each plant material and at each of the decomposition periods was tested. These were obtained from nine decomposition series replicated four times.

‡Toxicity rating was based on percentage inhibition of respiration of 6-day-old Harrow Velvet tobacco seedlings as compared with the buffer control: − =nontoxic: extracts which inhibit respiration of tobacco seedlings by less than 19%; +, ++, +++ =increasing orders of toxicity, producing inhibition of respiration of 20–40%, 41–60%, 61–95% respectively. (+) indicates instances where some of the extracts gave no inhibition of respiration while others in the same series gave inhibition values of 21–30%. (s) indicates cases where some of the extracts also produced stimulation of respiration.

all highly toxic and gave inhibition of respiration values of 65% or greater. Extracts in the pH 5.4 to 5.9 range were generally less toxic than those just mentioned although they still produced marked inhibiting effects on the respiration of tobacco seedlings. The relative toxicities of the extracts in this pH range were more variable, however, and other factors such as decomposition period and the type of plant residue which was added to the soil exerted some influence. For example, most of the extracts which were obtained at the 15- to 20-day decomposition period were highly toxic, while extracts obtained at an earlier or later period were moderately to weakly toxic. Throughout these tests most of the extracts with pH 6.0 or higher produced very little or no inhibiting effects on the respiration of tobacco seedlings, with one notable exception of tobacco which had been decomposing for 15 to 20 days. In this instance inhibiting effects on the respiration of tobacco seedlings was often produced by extracts with pH above 7.0, but toxicity was relatively low and respiration was inhibited by less than 40%. After the 15- to 20-day period the number of extracts in the highly toxic and acid range gradually decreased and by the 30th day more than half of the extracts were not toxic and had a pH above 6.0. These results are especially significant in view of the fact that the pH of soils to which no plant residues had been added did not vary from the initial range of 6.2 to 6.8 throughout the entire test period of 30 days and aqueous extracts obtained from these soils had no inhibiting effects on the respiration of tobacco seedlings.

Because of the consistent relationship between pH and toxicity, from the pH relatively accurate predictions could be made of the degree of toxicity resulting from the decomposition of a given plant residue in the soil. If, for instance, the pH of the soil after a certain decomposition period was below 6.0, it was highly probable that extracts of that soil would be toxic, whereas if it was above 6.0, the extracts were generally either weakly or nontoxic. Once attained, the toxin-producing capacity of each soil could be maintained almost indefinitely at a high level by the addition of plant residue and extracting at 3- to 5-day intervals.

The next series of tests was carried out to determine whether the inhibiting effects on the respiration of tobacco seedlings were due solely to the acidity of the extracts or to the presence in the extracts of toxic decomposition products. In these experiments, to keep them within manageable limits, comparisons were made only between extracts which preliminary tests indicated were either highly toxic or nontoxic. For this purpose extracts which in the other tests had reduced the oxygen uptake of tobacco seedlings by more than 80% were combined and provided the highly toxic solutions used in these and subsequent tests. Other extracts obtained from decomposition of similar plant residues but which did not inhibit respiration of tobacco seedlings formed the nontoxic solutions used in these and subsequent tests.

For this series of experiments four different test solutions were used, namely, 0.01 *M* phosphate buffer, fallow soil containing no decomposing plant residues, the highly toxic, and the nontoxic solutions described above. The pH of

each of the above test solutions was adjusted to pH values of 4.6 to 7.6 with HCl or NaOH. The effect of pH on the relative toxicity of the various solutions was determined in the usual manner. In Table IV there are summarized the results of an experiment in which the decomposition products of timothy were used as the sources of the toxic and the nontoxic test solutions. Similar results were also obtained when decomposition products of other crops were used as the test solutions.

TABLE IV

OXYGEN UPTAKE OF TOBACCO SEEDLINGS IN TOXIC AND NONTOXIC SOLUTIONS AS AFFECTED BY pH

	Type of solution			
pH	Buffer	Soil only	Toxic extract	Nontoxic extract
4.6	92*	94	8	90
4.9	110	106	8	104
5.2	124	120	6	136
5.5	122	118	10	134
5.8	122	120	7	138
6.1	126	127	10	140
6.4	129	128	41	128
6.7	125	122	68	135
7.0	122	126	75	138
7.3	127	129	81	142
7.6	133	135	98	146

*Oxygen uptake, in μl. of O_2, of 50 Harrow Velvet tobacco seedlings after 6 hours at 20° C. Each figure is an average of six determinations of four replicates each.

From the results in Table IV, it is evident that the inhibiting effects of the toxic solutions were not due to acidity, since varying the pH of the buffer solution or the other nontoxic solutions did not have an appreciable effect on the oxygen uptake of the tobacco seedlings. The wide range of applicability of the bio-assay test used in these studies was also evident and it was a relatively simple matter to evaluate accurately the toxic effects which were due solely to the acidity of the solutions and those which were due to the toxic decomposition products formed under acid conditions. Thus, it is clear that, although the toxic substances dealt with in this study are mainly produced under acid conditions, once they are formed they maintain their activity throughout the pH range of 4.6 to 6.1. At pH 6.1 and above, the toxic effects of these substances are gradually reduced as shown by the increase in the oxygen uptake of the tobacco seedlings. The toxic substances apparently were not completely destroyed, however, up to pH 7.6. In similar tests carried out with the nontoxic solutions change of pH from 7.6 to 5.2 did not induce toxicity. In most instances the oxygen uptake of the tobacco seedlings in such solutions was stimulated. As was expected extracts of soil which did not contain decomposing plant residues had no inhibiting effects on the respiration of tobacco seedlings and the oxygen uptake of tobacco seedlings

exposed to such extracts was inhibited only slightly at pH 4.6 to 4.9. This same degree of inhibition was also obtained in the buffer and the nontoxic solutions.

A few additional tests were carried out to determine whether the toxin-producing capacity and the degree of toxicity of a given soil could be altered by adjustment of the soil reaction. For this purpose a number of crocks were selected which contained crop residues that had been decomposing for 20 days but extracts of which were nontoxic to tobacco seedlings. The pH of these were adjusted from 6.7 to 5.0 with HCl. Similarly another series, containing similar crop residues at the same decomposition period except that the extracts in this case were highly toxic, was adjusted from pH 5.0 to 6.7 with NaOH. More plant residue was then added to both series of crocks and allowed to decompose for an additional 10 days. The pH of the contents of these two series of crocks was tested every 3 days adding HCl or NaOH when necessary to maintain their new pH values. At the end of the 10-day decomposition period, aqueous extracts were obtained from both series. It was found that the extracts from crocks which had been highly toxic initially gave rise to products which were only weakly toxic after their pH had been shifted to the less acid range. Extracts from the other series of crocks which had previously given rise to nontoxic products gave rise to toxic products following their adjustment to the more acid range.

Other experiments were carried out using soil which had an initial pH range of 5.0 to 5.4. The various plant materials were added to the soil in the usual manner. To one series of crocks, however, 10 g. of powdered calcium carbonate were added along with the appropriate plant residue. After a decomposition period of 20 days at 60–70° F., the pH and relative toxicity of each of

TABLE V

RELATIVE TOXICITY AS AFFECTED BY DECOMPOSITION OF THE VARIOUS PLANT RESIDUES IN SOILS WITH AND WITHOUT CALCIUM CARBONATE

Plant material added to soil	After 20-day decomposition period	
	pH	Oxygen uptake*
Soil only	5.4	129
Soil+$CaCO_3$	6.7	133
Timothy	4.9	12
Timothy+$CaCO_3$	6.2	146
Rye	5.7	42
Rye+$CaCO_3$	6.8	151
Corn	5.1	28
Corn+$CaCO_3$	6.4	141
Tobacco	5.8	66
Tobacco+$CaCO_3$	6.9	147

*O_2 uptake (in μl. of O_2 after 6 hours at 20° C.) of 50 Harrow Velvet tobacco seedlings. Each figure is an average of four determinations of four replicates each.

the extracts was determined in the usual manner. The results are presented in Table V, from which it is evident that, by maintaining the pH above 6.2 during the decomposition of the residue in question, it is possible to reduce markedly or prevent the formation of substances having inhibiting effects on the respiration of tobacco seedlings. This proved possible even with soils which at first had been quite acid.

The Effect of Aqueous Extracts of Undecomposed and of Partially Decomposed Plant Residues on the Respiration of Tobacco Seedlings

With respect to those toxic substances which may be liberated from the dead or dying plant organs which have been incorporated into the soil it has been shown (2, 3, 21, 23, 27) that many plants contain specific toxic components in certain of their organs which could be released into the soil solution by leaching or disorganization of the tissues. In other instances (11, 21, 28) it has been suggested that the toxicity is due to the intermediate reduction products formed when decomposition of plant residues takes place under certain soil conditions. With many plant residues both of these factors may be operating and it may not always be possible to distinguish between the two sources. Any method which would make it possible to determine relatively rapidly and with some accuracy whether the toxicity was due to unfavorable soil conditions or to specific chemical constituents of the plant in question would help considerably in attempts to modify the detrimental effects. The main purpose of these experiments, therefore, was to determine whether the bio-assay test for toxicity used in this study could also be adapted for this purpose, e.g., to determine whether the plants used in these studies contained specific toxic substances in their tissues or whether the toxic effects were mainly the result of decomposition of the residues under acid or high soil moisture conditions.

In the first series of tests comparisons were made between the effects on respiration of tobacco seedlings produced by exposure of the seedlings to aqueous extracts of soils in which the various plant residues had not or had just begun to decompose and those which had been exposed to extracts from similar soils in which the residues had been decomposing for 15 days. From the results presented in Fig. 2 it is evident that extracts from crocks in which the plant residues had not yet begun to decompose did not contain substances which induced inhibiting effects on the respiration of tobacco seedlings. In many of these tests the disorganization of the plant materials was effected still more completely by thorough maceration in the Waring blendor or by grinding to a fine powder in a Wiley mill before they were added to the soil, but here again no toxic effects were detected in the absence of decomposition.

In other experiments the various soils and plant materials were prepared in the usual manner except that they were placed in large Erlenmeyer flasks and plugged with cotton. One series of flasks was then partially sterilized by autoclaving for 40 minutes at 18 lb. pressure before incubating, whereas the other series was not autoclaved. After both series of flasks had been allowed to incubate for 15 days water extracts were obtained and the relative toxicity of the various extracts was determined in the usual manner. In this

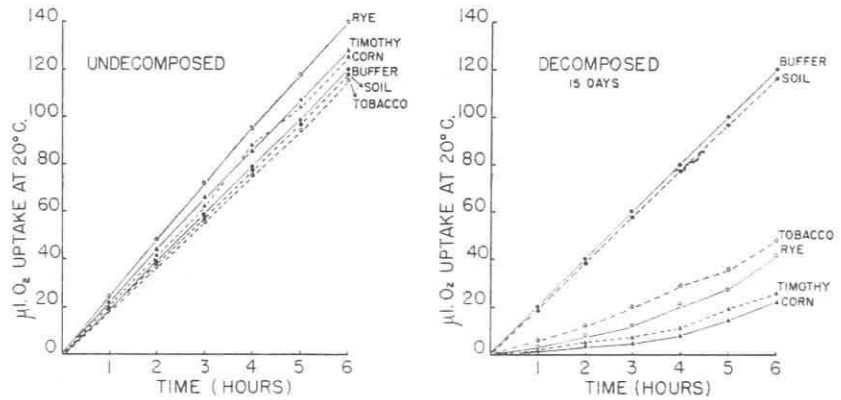

FIG. 2. Comparison of the O_2 uptake of 50 tobacco seedlings as affected by 16 hours' exposure to aqueous extracts of soil (pH 5.3–5.4) to which various plant materials had been added but which had not begun to decompose (undecomposed) and the O_2 uptake of similar seedlings exposed to extracts in which such plant materials had been decomposing for 15 days (decomposed 15 days). In each instance after the exposure period the seedlings were returned to phosphate buffer solution and the O_2 uptake measurements were carried out with the seedlings suspended in the buffer solution. The plant materials added to the soil are indicated in the graphs. Each Warburg vessel contained 2.2 ml. 0.01 *M* phosphate buffer solution (pH 5.3–5.8) and 0.2 ml. 20% KOH in the center well. The O_2 uptake of the buffer solution without the seedlings was negligible and no corrections were necessary.

instance, too, the effects on respiration were similar to those shown in Fig. 2 and autoclaving appeared to delay or inhibit the production of toxic substances. If, however, the autoclaved flasks were kept for periods longer than 15 days many of the soil organisms associated with toxin production apparently became re-established in sufficient numbers to produce some of the toxic decomposition products. Thus, for example, most of the extracts obtained after 25 days reduced the oxygen uptake by as much as 35%. The longer the interval between autoclaving and testing, the greater the degree of toxicity of the resulting extracts, timothy in each instance usually being the most toxic.

Color differences were also observed between extracts obtained from the autoclaved and the nonautoclaved series. After the 15-day period, water extracts of flasks from the autoclaved series were light brown in color while those from flasks which were not autoclaved were usually dark blue to black. The dark color appeared to be characteristic of most of the highly toxic extracts obtained throughout this study. The addition of NaOH (pH 8 to 10) to such extracts resulted in rapid flocculation and precipitation of the pigmented fraction. The toxicity of these extracts, however, was markedly reduced by this treatment. The resuspended precipitate too was only weakly toxic to tobacco seedlings and the toxic principle appeared to be destroyed by such a treatment.

Another difference which was noted between extracts from the autoclaved and the nonautoclaved series was associated with the pH. Whereas most of the extracts from the autoclaved series after the 15-day period were in the pH range of 6.4 to 7.1, those from the nonautoclaved series after the same period were in the 4.9 to 5.7 range. Readjustment of the pH of the extracts from the

autoclaved series to the pH range 4.9–5.7 or, conversely, readjustment of the extracts from the nonautoclaved series to 6.4–7.1 had little effect on their original toxicity.

In the final test of this series the various plant materials were macerated in the Waring blendor or were first air-dried and then ground to a fine powder in a Wiley mill. Water extracts of the plant materials were then obtained without the addition of soil. The pH of these extracts ranged between 6.7 and 7.5 and they were brown in color. After they were centrifuged and filtered the relative toxicity of each of these extracts was determined in the usual manner. Again, the results were similar to those in Fig. 2 and these extracts did not appear to have any inhibiting effects on the respiration of tobacco seedlings.

The results of these tests together with those presented earlier indicate that the tissues of the plants being dealt with in this study, namely, timothy, rye, tobacco, or corn, do not contain substances having inhibiting effects on the respiration of tobacco seedlings. Most of the toxic substances which were obtained appeared to have been formed during the decomposition processes. The total absence of specific toxic components in the tissues of the plants is not completely ruled out, however, since some of these substances might occur in certain tissues of these plants and not be released by leaching with water or maceration of the tissues. It is also possible that such toxic components might occur at stages of the plant maturity other than those tested. From the results presented it may be concluded, however, that the various toxic effects which have been obtained in this study are due mainly to the toxic products arising during the decomposition processes of the various crop residues under the experimental conditions described.

Rate of Penetration, Rapidity of Response, and Persistence of the Inhibiting Effects on Tobacco Seedlings by the Toxic Decomposition Products

The biological effectiveness of the toxic substances under present consideration and the magnitude of their harmful effects on plants would depend not only on their concentration in the soil solution but also on their rate of penetration or absorption by the roots, the persistence of the toxic effects produced by them, as well as on many other factors. Experiments were, therefore, undertaken to determine firstly how rapidly the inhibiting effects on the respiration of tobacco seedlings would become evident following their exposure to the action of the toxic decomposition products, and secondly to determine the persistence of the inhibiting effects upon removal of the seedlings from the toxic extracts.

The general procedures for these tests were similar to those used for the other respiration studies described. In these tests, however, the tobacco seedlings were not pre-exposed to the various extracts for the 16-hour period period prior to the determination of their respiration rates. The oxygen uptake of the seedlings was determined for the first hour in the 0.01 *M* phosphate buffer solution, then the various test extracts were added from the side

arms of the Warburg flasks and the oxygen uptake measurements were continued for an additional 8 hours with the seedlings exposed to the action of the various extracts and the buffer solution. Sterile tobacco seedlings were obtained in the usual manner and the test extracts used were those which, from previous tests, were known to be highly toxic. The Warburg flasks were prepared in the conventional manner with 50 tobacco seedlings per flask. Each Warburg flask contained 1.2 ml. of phosphate buffer solution in which the seedlings were suspended. One milliliter of the various test extracts was then placed in the side arms and alkali was placed in the center well. Thus each of the test extracts was diluted 1.2 to 1 when the two were finally combined in the flasks. The results are presented in Fig. 3 where the oxygen uptake of the tobacco seedlings under the influence of any of the extracts is given as the difference between the oxygen uptake of the flasks containing the seedling and the oxygen uptake of flasks containing the same extracts but without the seedlings. The results were not used, however, if the total oxygen uptake of these flasks without the seedlings exceeded 10 μl. of O_2 per flask during the total test period since this indicated that the extracts in these flasks had not been properly filtered and still contained some microorganisms.

From the results presented it will be noted that the substances present in some extracts were so potent that the inhibiting effects on the respiration of tobacco seedlings could be measured when the seedlings had been in the extracts for less than one hour. The severity of the inhibiting effects on respiration varied in accordance with the plant residue, decomposition products from

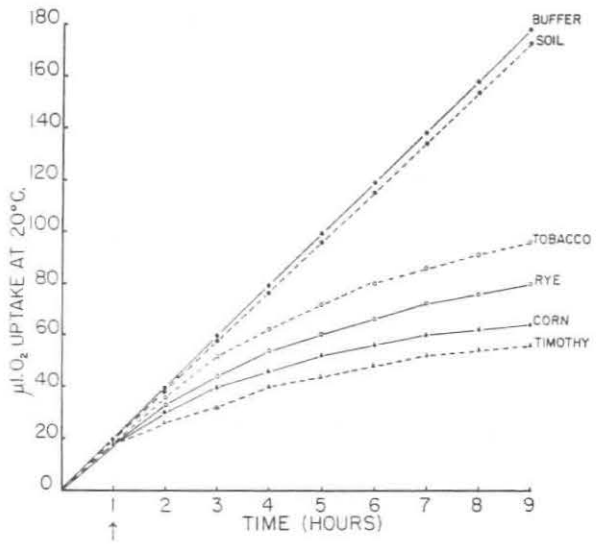

FIG. 3. Oxygen uptake of 50 tobacco seedlings in phosphate buffer solutions to which had been added aqueous extracts of soils in which the various plant materials indicated in the graph had been decomposing for 15 to 25 days. Each Warburg vessel contained 1.2 ml. of the buffer solution (pH 5.3–5.8) in which the seedlings were suspended, 0.2 ml. 20% KOH in the center well, and 1.0 ml. of the various test solutions (pH 5.3–5.8) which were added from the side arms after the 1st hour as indicated by the arrow. Corrections were made for the O_2 uptake of the test solutions without the seedlings.

timothy being the most toxic and those from tobacco showing the least toxicity. This could be due to the differences in the relative concentrations of the toxic components or it could indicate chemical differences between the substances produced during the decomposition of the various residues.

Experiments were next carried out to determine the persistence of the inhibiting effects produced by the 8-hour exposure period to the action of the various extracts. For this purpose the tobacco seedlings from each flask in the experiment above were carefully removed and kept separate. They were rinsed twice in sterile distilled water, returned to the Warburg flasks containing sterile phosphate buffer solution, and their oxygen uptake was again determined. Since this procedure required about two hours the seedlings remained in sterile distilled water for this period of time before their oxygen uptake was again determined. The results are presented in Fig. 4. It will be observed that there was little recovery from the injurious effects produced by the decomposition products of timothy or corn but almost complete recovery from the products of tobacco and rye. These differential rates of recovery and the irreversibility of the injurious effects produced by the decomposition products of some plant residues may be highly significant in connection with the relative severity and type of injury that these substances might induce on plants under field conditions.

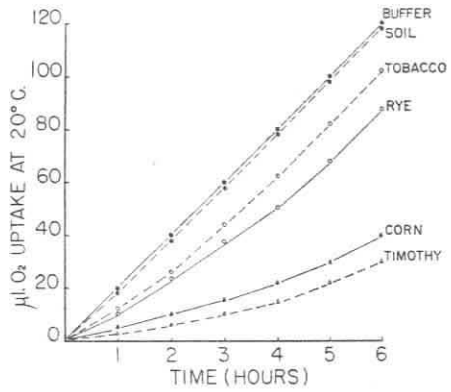

FIG. 4. Oxygen uptake of 50 tobacco seedlings in phosphate buffer. The seedlings were from the experiments described in Fig. 3 and had been exposed for 8 hours to decomposition products of the crops indicated. Each Warburg vessel contained 2.2 ml. 0.01 *M* phosphate buffer solutions (pH 5.3–5.8) and 0.2 ml. 20% KOH in the center well. The O_2 uptake of the buffer solution without the seedlings was negligible and no corrections were necessary.

Effect of the Various Toxic Extracts on the Roots of Tobacco Seedlings

In addition to the marked inhibiting effects on respiration of tobacco seedlings the substances arising during the decomposition of the various plant residues produced other effects. One of the more striking of these was their highly selective effects on the different regions of the root. When tobacco seedlings were placed in some of the extracts the apical meristem region soon turned dark brown (Fig. 6) whereas in other extracts the zone of elongation turned brown while the region of the apical meristem remained white (Fig. 7).

In tobacco seedlings placed in nontoxic extracts, in buffer solution, or in extracts of soil which did not contain decomposing plant residues both of these regions remained relatively white (Fig. 5). A more detailed examination of this phenomenon showed that the effect on the apical meristem was produced mainly by the highly toxic extracts which were obtained when the various plant residues had been decomposing in the soil for 15 to 25 days, while the effects on the region of elongation were obtained with extracts which were somewhat less toxic. In general, those extracts which reduced respiration by 60% or more also produced the browning of the apical meristem region and those which inhibited respiration by 25 to 60% produced the browning of the elongation zone and apparently produced no visible effect on the apical meristem. There were a few exceptions, especially timothy decomposition products which almost exclusively affected the apical meristem and those of rye which affected principally the elongation region. In no instance was browning of either region produced by extracts which did not inhibit respiration.

Microscopic examination of crushed tissue mounts showed that the affected cells of the apical meristem were filled with disorganized masses of brown granular and amorphous material while there was no discoloration in the root cap or the region above the apical meristem. Parallel examinations of roots where the region of elongation was brown showed that the brown pigment was

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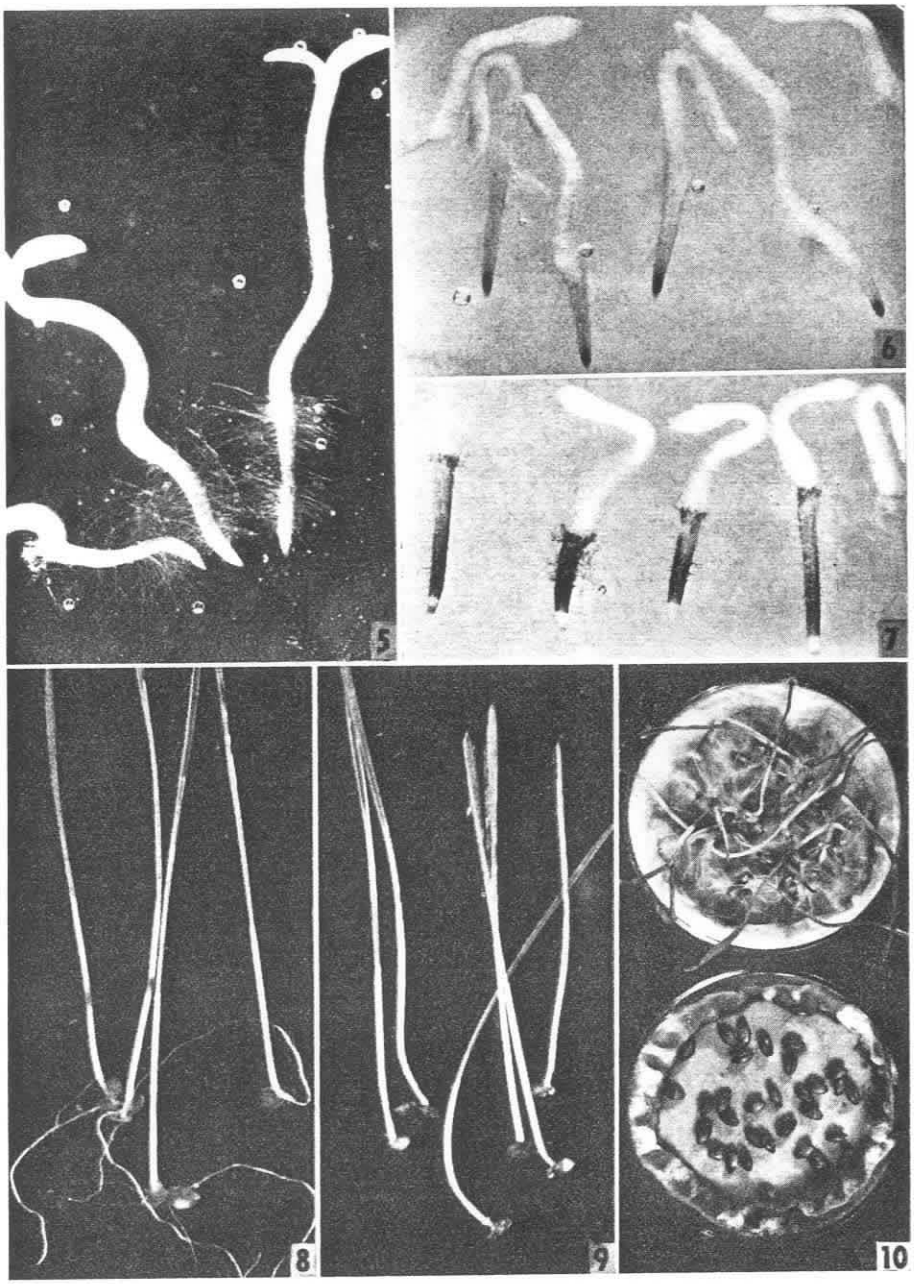
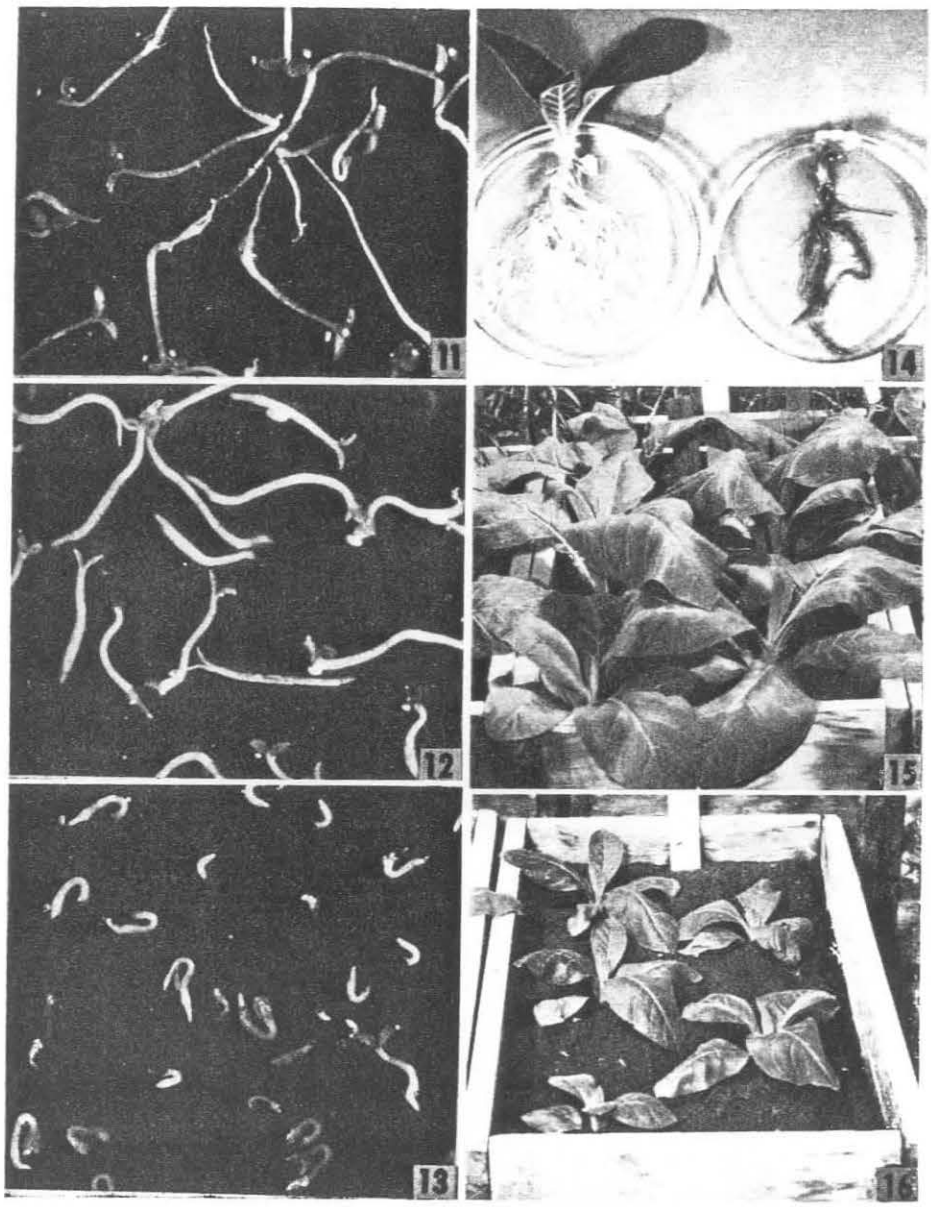
FIGS. 5 to 7. These figures illustrate a few of the more striking effects which were observed when tobacco seedlings were exposed to toxic extracts obtained from the decomposition of the various plant residues in the soil. Initially the seedlings used in these tests were identical in appearance, all having been taken from the same composite sample on the 6th day of germination. When such seedlings were exposed for 12 hours to buffer solution or to aqueous extracts of soil which did not contain decomposing plant residues they remained healthy with white roots and numerous root hairs (Fig. 5). When similar seedlings were exposed for the same period of time to aqueous extracts of soil in which residues from timothy, corn, rye, or tobacco had been decomposing for various periods of time, their growth and root hair development was greatly inhibited. The roots were especially sensitive to these substances and visible browning and necrosis of root cells often occurred within 1 hour after exposure. The toxic products were selective in their action and certain of these extracts affected only the cells of the meristematic region (Fig. 6). Browning of this region was induced most frequently by decomposition products from timothy and by extracts which in the other tests had reduced respiration by over 60%. Other extracts, especially those from the decomposition of rye, affected only the cells of the elongation region and the cells of the meristem region remained white (Fig. 7).

FIGS. 8 and 9. Effects similar to those described above were obtained when timothy seedlings were used in place of tobacco and while normal growth and root development were obtained when timothy seedlings were exposed to extracts of unamended soil (Fig. 8) marked inhibition and root injury were obtained when they were exposed to extracts containing toxic decomposition products (Fig. 9). As in the case of tobacco, although all parts of the seedlings were in contact with the toxic extracts the visible injurious effects appeared to be restricted mainly to the region of the root and in most instances root development was partially or completely inhibited (Fig. 9).

FIG. 10. The toxic substances formed during the decomposition of the various plant residues were not specific for tobacco and the same effects were produced when either tobacco, timothy, or barley seed was exposed to their action. Thus when barley seed was placed on filter paper which previously had been soaked in the toxic extracts no germination occurred after 7 days, while those placed on filter paper soaked in water had all germinated by this time. The seed used in this instance had not been surface sterilized and by the 7th day the filter paper and many of the germinating seedlings were covered by fungal growth (top Petri dish); no such fungi were observed on seed which had been exposed to the toxic extracts (bottom Petri dish).

PLATE I





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mainly concentrated in the cell walls, with the epidermal cells being most affected and the discoloration gradually decreasing toward the center of the root.

Other experiments showed that the effect on the apical meristem was quite rapid, that browning could be observed when seedlings were placed for less than one hour in extracts which produced this effect, and that the effect was irreversible when the seedlings were removed from the toxic extracts. Where the browning effect was on the region of elongation discoloration could be noted only in about four to six hours after the seedlings had been exposed to the extracts and again appeared to be irreversible.

All the extracts that possessed other toxic properties also inhibited root hair formation and root hairs were much more abundant on seedlings which had been exposed to extracts of soil only (Fig. 5) than those which had been exposed to the highly toxic extracts containing decomposing plant residues (Figs. 6 and 7). It was also noted that although all parts of the seedlings were in contact with the toxic extracts the visible injurious effects appeared to be restricted mainly to the regions of the root.

In parallel tests carried out with tobacco and timothy seedlings and with excised corn root tips it was found that while timothy seedlings were affected in the same manner as the tobacco by the various extracts, the excised corn root tips, on the other hand, did not show the browning of either the apical meristem or the region of elongation even though their respiration was inhibited.

Effect of Decomposition Products of Timothy, Rye, Tobacco, and Corn on Germination and Growth of Tobacco

Although the respiration of tobacco seedlings was used as the principal assay method for evaluating the relative toxicity of the substances under consideration in these studies, their effects on the germination and growth of

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FIGS. 11 to 13. The effect of the various toxic extracts on germination and growth of tobacco is illustrated. The results were taken after the 7th day and good germination and growth was obtained when the seed had been placed on filter paper previously soaked in water (Fig. 11). Similar and often slightly better germination and growth was obtained when the seed was placed on filter paper soaked in aqueous extracts of unamended soil. When tobacco seed was placed on filter paper soaked in extracts containing decomposition products of rye or tobacco the germination was delayed and growth was inhibited (Fig. 12). Marked inhibition of germination and growth was obtained when the tobacco seed was exposed to decomposition products of timothy or corn (Fig. 13); in many instances germination of tobacco, timothy, or barley seed was completely inhibited by such extracts.

FIG. 14. Severe wilting and rotting of roots of older tobacco plants were also readily induced by many of the toxic decomposition products. These injurious effects were readily demonstrated in plants growing in nutrient culture in quartz sand. The plant on the right had received one application of the toxic decomposition products from timothy. Severe wilting was obtained 24 hours after the application and discoloration and rotting of the roots was obtained 3 days after the application of the toxic extracts.

FIGS. 15 and 16. Stunting of tobacco plants was obtained in the greenhouse when materials from timothy, rye, tobacco, or corn plants were added to flats of soil and allowed to decompose for 21 days before tobacco was planted. Good growth was obtained in flats to which no plant residue had been added (Fig. 15). Plants growing in flats to which timothy or corn residue had been added were severely stunted (Fig. 16). The stunting was less severe in flats to which rye or tobacco residue had been added.

TABLE VI

EFFECT ON GERMINATION AND GROWTH OF TOBACCO AFTER 7 DAYS' EXPOSURE TO THE VARIOUS EXTRACTS

Type of test extract	Decomposition period (days) at 60–70° F.						
	0	5	10	15	20	25	30
Water (check)	225*	210	195	210	205	210	203
Soil	240	215	202	242	237	245	208
Soil+timothy	215	82	60	55	65	100	85
Soil+rye	227	110	72	82	127	107	130
Soil+corn	213	123	135	65	85	68	98
Soil+tobacco	232	164	142	105	132	205	200

*Total length in mm. of 25 tobacco seedlings after 7 days' incubation at room temperature (70–80° F.). Each figure represents the averages of eight different decomposition series in which tests with each extract were replicated four times.

tobacco, barley, and timothy were also determined as a check on the reliability of the basic technique and to determine the range of activity of these substances. The method was described in some detail earlier and most of the extracts on which respiration studies were carried out were also tested by this method.

The results are summarized in Table VI from which it will be noted that the relative toxicity of the various extracts as determined by this method was reasonably similar to that in which respiration was used as the test response. In this method the relative toxicity is shown as the length in millimeters of 25 germinating tobacco seedlings after 7 days. Thus, the extracts which were highly toxic as determined by the effects on respiration were also the most toxic as determined by the latter method. It appears, therefore, that the substances which are produced during decomposition of the various plant residues can affect respiration, germination, and growth. In these tests no germination of tobacco seed occurred even after 15 days in extracts which had inhibited respiration by 70% or more. In other extracts which had less pronounced effects on respiration the effects on germination and growth were also less pronounced and ranged from germination after only 3 days followed by rapid growth to rapid germination followed by slow growth with necrosis of the radicles and to delayed germination with little or no further growth. As with respiration, these effects too were influenced by the type of plant residue added to the soil, the decomposition period, as well as by the other factors dealt with previously. Again decomposition products of timothy were most toxic while those of tobacco showed the least toxicity.

Additional Physical and Chemical Properties of the Toxic Decomposition Products

A few additional tests were carried out with the highly toxic extracts to determine their specificity, solubility, stability to heat and in storage, and other properties.

To test the specificity of the toxic extracts a series of tests were carried out to determine whether the toxic effects on germination and growth of tobacco

seed would also be produced when barley or timothy seed were used. These tests were carried out simultaneously and the effects on the three types of seed by the various toxic extracts were compared. Comparisons were also made of the effects on the respiration of tobacco seedlings and on excised corn root tips and almost identical results were obtained with each. From the results obtained it appears that the toxic substances present in the various extracts were relatively nonspecific in their action, affecting tobacco, timothy, and barley in approximately the same manner (see Figs. 5–10).

During many of the tests it was noted that all of the toxic extracts also had some antifungal properties. This was most clearly illustrated in tests in which the seed used had not been surface-sterilized. In such cases it was noted that when the seeds were placed on filter paper soaked in the nontoxic extracts or in water they were soon covered with the fungi which normally are found on the surface of these seeds. Germination and growth of tobacco and barley seed did not appear to be appreciably affected by most of these organisms during the 7-day test period. Seed placed on filter paper soaked in the toxic extracts remained completely free of fungi even though they contained seed from the same sample (Fig. 10). Tests to determine the species of fungi which could be inhibited by these extracts have not been completed.

In preliminary isolation, purification, and concentration tests it was found that the toxic substances were soluble in water and insoluble in petroleum ether. They were stable in acid and were most active in the acid range pH 4.5 to 5.8. They were inactivated by alkali and did not regain their original activity when readjusted to the acid range. These substances were also found to be heat stable. Boiling for ½ hour or autoclaving for 20 minutes at 16 lb. pressure at pH of 4.0 to 5.5 did not appreciably reduce their toxicity. The toxicity of the extracts was not affected on prolonged storage at 1° to 3° C. provided they were covered by a layer of toluene. The toxicity was somewhat reduced after the extracts had passed through activated charcoal.

Greenhouse Experiments

A few preliminary experiments were carried out in the greenhouse with older tobacco plants to determine whether certain of the toxic effects could be observed under greenhouse conditions. In one series of tests, which will be described briefly, the various plant residues were added to a Fox sandy loam soil obtained from the Delhi tobacco growing area. This soil was placed in deep flats, well fertilized, and the plant material was allowed to decompose for 21 days under normal moisture conditions. There were four replicates for each of the plant residues used and flats of the same soil to which no plant residues had been added served as the controls. After a 21-day decomposition period five tobacco plants each 6 in. in height were planted in each of the flats and results were determined 15 days after planting. Severe stunting was obtained in flats to which timothy or corn had been added. Less stunting was obtained in flats where rye or tobacco had been added and good growth was obtained in flats which did not contain crop residues (Figs. 15 and 16).

In other experiments tobacco was grown in quartz sand in which some of the plants received nutrient solution while others received both nutrient solution and the highly toxic extracts. In many instances severe wilting was obtained 24 hours after the application of the toxic extracts. The roots of the plants receiving the highly toxic extracts had also turned black and rotted within 3 days after the application of the extracts (Fig. 14). Applications of the toxic extracts were also made to well-established tobacco plants growing in soil. In this instance, however, some wilting was observed only after the fifth application, suggesting that much of the toxic component was adsorbed by the soil colloids and much larger quantities of the extracts had to be added before some plant response became evident. More comprehensive greenhouse and field experiments will be necessary, however, before the precise significance of the various toxic effects observed here under laboratory conditions can be fully evaluated and related to conditions in the field.

Discussion

The present studies have shown that substances possessing phytotoxic properties may be formed during the decomposition of plant residues in the soil. This confirmation of the production of phytotoxic compounds during the decomposition of residues is of special interest because of the suspected importance of such compounds in the etiology of certain root rots (10, 13, 17, 18, 24). It is with these toxic compounds, therefore, that the present studies have been primarily concerned. An attempt has been made to elucidate some of the factors concerned in their formation, to evolve suitable methods for their assay, and to study some of the harmful effects that such substances have on plants in the absence of other causal factors.

Since it was quite evident that most studies dealing with the question of whether or not decomposition of plant residues in the soil can give rise to substances toxic to plants have been seriously handicapped by the lack of reliable and rapid assay methods, concerted efforts were made to approach the problem on a more precise qualitative and quantitative basis. With this purpose in mind the possibility of adapting the Warburg respirometer for the purpose of studying certain phases of the problem was thoroughly explored. By using inhibition of respiration as an index of toxicity it was possible to determine some of the conditions under which the decomposition of plant residues in the soil gave rise to phytotoxic by-products and to obtain an accurate measure of the relative toxicity of such substances. Thus, certain relationships between the nature of plant residue added to the soil, the decomposition conditions, and the degree of toxicity of the resulting products could be readily determined. If on wider application and further study this bio-assay method should continue to be as satisfactory as in the current investigation it would offer considerable promise in overcoming many of the difficulties encountered in such studies.

Since these have been laboratory experiments, the interpretation as to the possible significance of the results presented must be viewed in the light of

this obvious limitation. Whether the toxic decomposition products obtained in this study can also be produced under field conditions or affect the plants in the manner indicated in such a complex system as the soil environment cannot be definitely stated at this time. In view of the constant occurrence of plant residues as one of the normal soil constituents, however, and since the toxic substances dealt with in this study were obtained quite readily under conditions of decomposition which might also pertain at certain times in the field, the possibility that similar substances may be produced and released into the soil solution is not too remote. It may be true that, although conditions most favorable for the production of toxic products are most likely to occur in poorly drained and acid soils, the production of such substances is not limited to such soils. Since in these experiments they could be produced relatively readily in all of the soils tested, many of which initially were only weakly acid and only temporarily saturated, it is possible that their occurrence may be more common than is generally realized. It is possible, therefore, that toxic decomposition products may occur in soils and under conditions which when considered as a whole would not appear to favor their production. In such instances these substances are likely to be produced in localized areas where the concentration of plant residues, water content, pH, and other factors are favorable for the type of breakdown leading to toxin formation. There is no doubt that most of these substances, no matter how toxic they are, eventually disappear from the soil (2). Regardless of the period of existence of such compounds, however, because of their marked toxicity and rapidity of action they could, nevertheless, induce important physiological and morphological changes in the plant. Owing to the nonspecific nature of their action and the difficulty of their detection, many of the harmful effects would often be attributed to some other more obvious causal factors. By using the more precise and sensitive assay methods, however, it has been clearly demonstrated that many of these substances can exert injurious effects in the absence of other causal factors. It is significant, too, that the roots appear to be especially sensitive to these products. It may be possible that the index used to demonstrate and evaluate toxicity is not a true reflection of the over-all phytotoxic nature of the products involved, since the significance of many of the respiratory changes in plants is not clearly understood (1, 7). There is a strong likelihood, however, that any condition which gives rise to a reduction in the respiratory activity is also likely to result in some retardation of all the physiological functions, with the corresponding inhibiting effects on many of the processes associated with growth and development. Combined with the inhibitory effects on respiration the same extracts induced marked injury to root cells and either completely inhibited or retarded germination and growth. These results suggest, therefore, that in addition to the more readily visible injurious effects many of the toxic decomposition products are also likely to exert less obvious but perhaps more significant changes in the living plant. Many of these are likely to be expressed indirectly as a deficiency, a stunting, or more commonly a root-rot condition produced by organisms whose pathogenicity often cannot be conclusively demonstrated.

Therefore, in view of their ability to produce such a broad range of injurious effects in the absence of other causal factors, it is highly probable that toxic decomposition products may be performing a significant role in the soil not only as primary causal and initiating agents of certain root rots but also in predisposing the plant, especially the root system, to invasion by organisms not normally regarded as pathogenic. In the light of the data presented and other information available (12, 13, 24, 29) it is evident that in a consideration of almost any root-rot disease of plants the important role of such products in the disease complex cannot be overlooked.

Acknowledgments

The technical assistance of Mr. H. J. Thorpe in the preparation of the photographs and charts is gratefully acknowledged.

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